

WHAT IS CLAIMED IS:

1 1. A method for separating an intact NP probe from a phosphate
2 detectable moiety, said method comprising:

3 a) providing a sample comprising an intact NP probe with a detectable
4 moiety attached thereto, whereupon an enzymatic cleavage of said intact NP probe, which
5 produces a phosphate detectable moiety, said phosphate detectable moiety carries a molecular
6 charge which is different than the molecular charge of said intact NP probe; and

7 b) applying an energy field to said sample, thereby separating said
8 phosphate detectable moiety from said intact NP probe.

1 2. The method according to claim 1, wherein said intact NP probe is a
2 charge-switch nucleotide phosphate probe having a detectable moiety on a terminal
3 phosphate.

1 3. The method according to claim 2, wherein said charge-switch
2 nucleotide phosphate is a nucleotide triphosphate (NTP) having a γ -phosphate with a
3 detectable moiety attached thereto.

1 4. The method according to claim 3, wherein said γ -phosphate with a
2 detectable moiety attached thereto is a γ -phosphate with a fluorophore attached thereto.

1 5. The method according to claim 1, wherein said intact NP probe is
2 incorporated on a primer strand hybridized to a target nucleic acid using a polymerase,
3 thereby releasing said phosphate detectable moiety.

1 6. The method according to claim 1, wherein said polymerase is
2 immobilized.

1 7. The method according to claim 1, wherein said energy field is an
2 electric field.

1 8. The method according to claim 7, wherein said electric field is a first
2 electric field applied in a transverse direction and a second energy field is applied in an axial
3 direction.

1 9. The method according to claim 8, wherein said second energy field
2 applied in said axial direction is a pressure field.

1 10. The method according to claim 1, wherein the charge of said phosphate
2 detectable moiety is greater than said intact NP probe.

1 11. The method according to claim 1, wherein the charge of said phosphate
2 detectable moiety is less than said intact NP probe.

1 12. The method according to claim 1, wherein the charge of said phosphate
2 detectable moiety is opposite in sign compared to said intact NP probe.

1 13. The method according to claim 1, further comprising c) detecting said
2 phosphate detectable moiety.

1 14. The method according to claim 13, wherein said detection is via a
2 charge coupled device (CCD) camera.

1 15. The method according to claim 13, wherein said detection is via a dye-
2 impregnated polymeric coating on optical fiber sensor.

1 16. The method according to claim 13, wherein said detection is via a
2 photodiode.

1 17. The method according to claim 13, wherein said detection is via a
2 blockade current.

1 18. A method for identifying an intact charge-switch nucleotide phosphate
2 (NP) probe, said method comprising:

3 a) contacting a sample comprising said intact charge-switch NP probe
4 with an enzyme to produce a phosphate detectable moiety; and

5 b) applying an electric field to said sample, wherein said phosphate
6 detectable moiety migrates to an electrode differently than said intact charge-switch NP
7 probe.

1 19. The method according to claim 18, wherein said electrode is an anode.

1 20. The method according to claim 18, wherein said electrode is a cathode.

1 **21.** The method according to claim **18**, wherein either said intact NP probe
2 has a positive molecular charge, or wherein upon cleavage of said phosphate detectable
3 moiety, said phosphate detectable moiety carries a positive charge relative to said intact NP
4 probe.

1 **22.** The method according to claim **18**, wherein said enzyme is selected
2 from the group consisting of a DNA polymerase, a DNA dependent RNA polymerase, a
3 reverse transcriptase, a phosphodiesterase and a phosphatase.

1 **23.** The method according to claim **18**, wherein said intact charge-switch
2 NP probe is a member selected from the group consisting of a nucleotide diphosphate, a
3 deoxynucleotide triphosphate (dNTP), and a nucleotide triphosphate (NTP).

1 **24.** The method according to claim **23**, wherein said deoxynucleotide
2 triphosphate (dNTP) is a member selected from the group consisting of deoxyadenosine
3 triphosphate, deoxycytosine triphosphate, deoxyguanosine triphosphate deoxythymidine
4 triphosphate and deoxyuridine triphosphate.

1 **25.** The method according to claim **18**, wherein said phosphate detectable
2 moiety is a pyrophosphate with a fluorophore moiety attached thereto.

1 **26.** The method according to claim **25**, wherein upon cleavage of said
2 pyrophosphate fluorophore moiety, said pyrophosphate fluorophore moiety carries a positive
3 charge relative to said intact NTP probe.

1 **27.** The method according to claim **18**, wherein said intact NP probe has a
2 positive charge.

1 **28.** The method according to claim **18**, wherein said intact NP probe has a
2 negative charge.

1 **29.** An intact charge-switch nucleotide phosphate (NP) probe, wherein,
2 upon enzymatic cleavage of said intact charge-switch NP probe to produce a phosphate
3 detectable moiety, said phosphate detectable moiety migrates to an electrode, and intact
4 charge-switch NP probe migrates to the other electrode.

1 **30.** The intact charge-switch NP probe according to claim **29**, wherein
2 either said intact NP probe has a positive molecular charge, or wherein upon cleavage of said
3 phosphate detectable moiety, said phosphate detectable moiety carries a molecular positive
4 charge relative to said intact NP probe.

1 **31.** The intact charge-switch NP probe according to claim **29**, wherein said
2 charge-switch NP probe is a nucleotide triphosphate (NTP); and wherein said phosphate
3 detectable moiety is a pyrophosphate with a fluorophore moiety attached thereto.

1 **32.** The intact charge-switch NP probe according to claim **29**, wherein said
2 intact NTP probe has a positive charge.

1 **33.** The intact charge-switch NP probe according to claim **31**, wherein
2 upon cleavage of said phosphate detectable moiety as a pyrophosphate fluorophore moiety,
3 said pyrophosphate fluorophore moiety carries a positive charge relative to said intact NTP
4 probe.

1 **34.** The intact charge-switch NP probe according to claim **29**, wherein said
2 NTP probe is a member selected from the group consisting of a deoxynucleotide triphosphate
3 (dNTP), and a nucleotide triphosphate (NTP).

1 **35.** The intact charge-switch NP probe according to claim **34**, wherein said
2 NTP probe is a deoxynucleotide triphosphate (dNTP).

1 **36.** The intact charge-switch NP probe according to claim **35**, wherein said
2 deoxynucleotide triphosphate (dNTP) is a member selected from the group consisting of
3 deoxyadenosine triphosphate, deoxycytosine triphosphate, deoxyguanosine triphosphate
4 deoxythymidine triphosphate and deoxyuridine triphosphate.

1 **37.** The intact charge-switch NP probe according to claim **34**, wherein
2 said nucleotide triphosphate (NTP) is a member selected from the group consisting of
3 adenosine triphosphate, cytosine triphosphate, guanosine triphosphate and uridine
4 triphosphate.

1 **38.** The intact charge-switch NP probe according to claim **31**, wherein
2 said fluorophore moiety is attached to said terminal phosphate via a linker.

1 **48.** The method according to claim **45**, wherein said energy field is an
2 electric field.

1 **49.** The method according to claim **48**, wherein said electric field is a first
2 electric field applied in the transverse direction and a second electric field applied in the axial
3 direction.

1 **50.** A method for sequencing a nucleic acid, said method comprising:
2 providing a target nucleic acid, a polymerase priming moiety, a polymerase,
3 and a plurality of intact NP probes;
4 mixing said target nucleic acid, said polymerase priming moiety, said
5 polymerase and said plurality of NP probes under conditions permitting target dependent
6 polymerization of said plurality of NP probes, such conditions which are capable of providing
7 a time sequence of a plurality of phosphate detectable moieties;
8 separating by charge said plurality of phosphate detectable moieties from said
9 plurality of intact NP probes; and
10 detecting over time said plurality of phosphate detectable moieties to provide a
11 sequence of said target nucleic acid.

1 **51.** The method according to claim **50**, wherein said primer moiety is a
2 hairpin loop.

1 **52.** The method according to claim **50**, wherein said plurality of phosphate
2 detectable moieties independently selected from the group consisting of PPI-Dye, a terminal
3 phosphate fluorophore moiety, a detectable moiety, charged groups, electrically active
4 groups, reporter groups, and combinations thereof.

1 **53.** The method according to claim **52**, wherein said phosphate fluorophore
2 moiety is a used for a member selected from the group consisting of one-color sequencing,
3 two-color sequencing, three-color sequencing, four-color sequencing and combinations
4 thereof.

1 **54.** The method according to claim **50**, wherein said polymerase is
2 immobilized in single molecule configuration.